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Amino Acid-Based Bioanalogous Polymers. Synthesis, and Study of Regular Poly(ester amide)s Based on Bis(α-amino acid) α,ω-Alkylene Diesters, and Aliphatic Dicarboxylic Acids

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ABSTRACT: The purpose of this research was to synthesize new regular poly(ester amide)s (PEAs) consisting of nontoxic building blocks like hydrophobic α-amino acids, α,ω-diols, and aliphatic dicarboxylic acids, and to examine the effects of the structure of these building block components on some physico-chemical and biochemical properties of the polymers. PEAs were prepared by solution polycondensation of di-*p*-toluenesulfonic acid salts of bis-(α-amino acid) α,ω-alkylene diesters and di-*p*-nitrophenyl esters of diacids. Optimal conditions of this reaction have been studied. High molecular weight PEAs (M_w = 24,000–167,000) with narrow polydispersity (M_w/M_n = 1.20–1.81) were prepared under the optimal reaction conditions and exhibited excellent film-forming properties. PEAs obtained are mostly amorphous materials with T_g from 11 to 59°C. α-Chymotrypsin catalyzed *in vitro* hydrolysis of these new PEA substrates was studied to assess the effect of the building blocks of these new polymers on their biodegradation properties. © 1999 John Wiley & Sons, Inc. J Polym Sci A: Polym Chem 37: 391–407, 1999

Keywords: α-amino acids; biodegradable polymers; regular poly(ester amide)s; α-chymotrypsinolysis

INTRODUCTION

One of the most important criteria for designing degradable polymers for biomedical applications is biocompatibility of the polymers and their degradation products. Polymers that are made of naturally occurring building blocks are preferable materials for biomedical applications because their degradation products are nontoxic and can be metabolized properly by living tissues.[1,2] Considering this point of view, α-amino acid derived polymers—synthetic analogues of proteins—could be one of the most promising candidates.

Conventional poly(α-amino acid)s like the AB-type Nylon-2 have been extensively investigated by many investigators,[3–5] and have proved to be relatively less suitable as resorbable biomaterials for many reasons given by Nathan and Kohn.[3] The reported major shortcomings of this type of poly(α-amino acid)s are: (a) difficult and costly manufacturing processes, such as expensive to produce in larger quantities from extremely unstable and expensive compounds (*N*-carboxyanhydrides); (b) insolubility in common organic sol-

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vents and thermal degradation upon melting process; (c) the rates of biodegradation under physiological conditions are often too slow to be useful in biomedical engineering; and (d) immunogenicity of poly(α-amino acid)s that increases with increasing molecular complexity (e.g., branching) and the number of different amino acid residues presented in the copolymer. It is also known that the immunogenicity of an amino acid-derived polymer is strongly dependent on structure and conformation.[6] Therefore, it is expected that heterochain polymers composed of α-amino acids and backbone linkages other than peptide bonds would show at least a lower immunogenicity when comparing with conventional poly(α-amino acid)s.

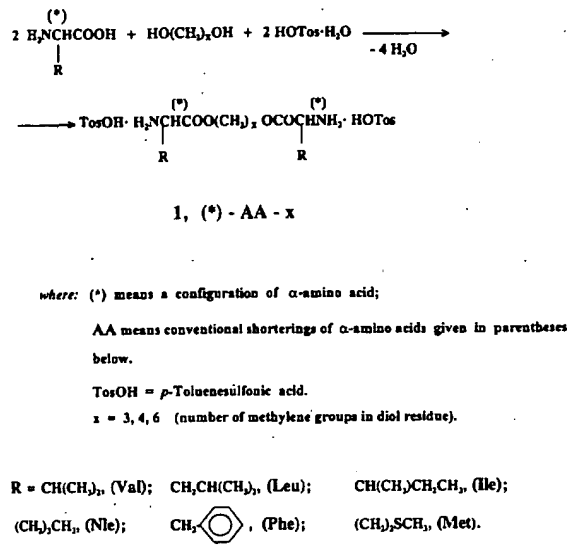
In the light of all the aforementioned polydepsipeptides, regular copoly(α-ester α-amide)s, which combine many useful properties of poly(α-hydroxy acid)s and poly(α-amino acid)s, could be considered as attractive resorbable biomaterials. However, the preparation of polydepsipeptides by solution polycondensation of corresponding di, tri, or higher depsipeptides,[7–11] is too complex and expensive. An alternative way through cyclic monomers like morpholine-2,5-dions[12–16,19–21,23] also comprises some drawbacks. First, there are limited available synthetic routes and low yields (~ 30% based on α-amino acid) of monomers like morpholine-2,5-dions. Second, the formation of low molecular weight oligomers or polymers with unfavorable mechanical properties has been observed in some cases. Third, the severe reaction conditions by ring-opening melt polymerization (or copolymerization with lactones) may cause undesirable side reactions and unexpected chemical units in the polymer backbone. Because purity of materials is essential for biomedical applications, any unexpected byproducts from side reactions during polymer synthesis and the use of toxic organotin catalysts should be eliminated or minimized.

It seems to us, therefore, that a better approach would be the use of AA-BB type amino acid based bioanalogous polymers (AABBPs) containing various types of linkages in the backbones and prepared mainly by solution polycondensation under mild conditions without the usage of any toxic catalyst. It is very important to recognize that synthetic possibilities of this approach are virtually unlimited. This diversity in synthesis routes permits us to manufacture polymers having a wide range of material properties at a reduced cost.

We have studied the synthesis and properties of a variety of AABBPs since the early 1980s.[17–27] Within the framework of these investigations, we have recently reported the synthesis and some *in vitro* studies of regular poly(ester amide)s (PEAs) based on bis(L-phenylalanine) α,ω-alkylene diesters and adipic acid. We found that these poly(ester amide)s underwent biodegradation by the action of specific enzymes like α-chymotrypsin, and the rate of this enzymatic biodegradation increased with the length of the polymethylene chain of the diol used. A strong spontaneous enzyme immobilization on the surface of solid PEAs had also been observed. This immobilized enzyme degraded the polymeric surface at a nearly constant rate, and rapidly discharged degradation products into the surrounding environment. An accelerated biodegradation of biodegradable polymers like polyglycolide and PEAs has been suggested to be able to promptly activate macrophages that would, in turn, produce the required growth factors and other biochemicals for an accelerated wound healing.[28–30]

Our findings of the properties of regular PEAs permit us to seriously consider them as a new class of promising biomaterials for many biomedical applications ranging from surgical implants like vascular grafts, nerve guidance tubes, absorbable bone plates, pins and screws, surgical meshes and temporary artificial skin for burn treatment to drug delivery devices. In addition, these PEAs may be used as new substrates in enzymology as well as for the study of the pharmacological and immunologic activities of α-amino acid derived polymers.[31] This diversity in biomedical applications would certainly require a wide variety of mechanical, physico-chemical, and biochemical properties of polymers. In the case of AABBPs like regular PEAs, such a wide range of properties could be easily achieved by varying three components in the building block of the macromolecular backbone during synthesis: α-amino acid, diol, and dicarboxylic acid. This additional advantage would make this class of heterochain biodegradable polymers the prominent candidates for our pursuing of better biomaterials.

In this article, the synthesis of regular PEAs consisting of nontoxic building blocks like hydrophobic α-amino acids, α,ω-diols, and aliphatic dicarboxylic acids was performed to examine the effects of the structure of these building-block components on some physico-chemical properties of the polymers. The *in vitro* enzyme-catalyzed



Scheme 1. Synthesis of di-*p*-toluenesulfonic acid salts of Bis (α-amino acid α,ω-alkylene diester, **1**.

biodegradation of the newly synthesized PEAs was also studied to assess the effect of different building blocks of PEAs on their biodegradability.

EXPERIMENTAL

Materials

The new PEAs were synthesized from three building blocks: α-amino acids, diols, and fatty dicarboxylic acids. Six α-amino acids used in this study were L-Valine (Val), L-Leucine (Leu), L-Isoleucine (Ile), DL-Norleucine (Nle), L- and DL-Phenylalanine (Phe), and DL-Methionine (Met). The three diols were 1,3-propanediol, 1,4-butanediol, and 1,6-hexanediol, and the two dicarboxylic acyl chlorides were adipoyl and sebacoyl. Other chemicals and solvents were *p*-toluenesulfonic acid monohydrate, *p*-nitrophenol, *N,N*-dimethylacetamide, *N*-methyl-2-pyrrolidinone, benzene, nitrobenzene, ethylacetate, acetone, acetonitrile, chloroform, triethylamine, *N*-methylmorpholine, *N,N,N',N',*-tetramethylethylenediamine, and pyridine. All the solvents and the tertiary amines were purified by standard methods before use, while the others chemicals like α-amino acids, diols, and dicarboxylic acyl chlorides were purchased from Aldrich and used without further purification.

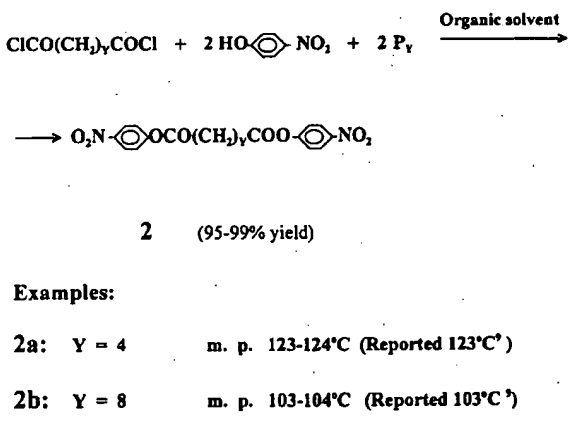
α-Chymotrypsin from bovine pancreas with activity 50–60 units per mg was purchased from Fluka and used for the *in vitro* biodegradation study. The definition of "unit" is that one unit hydrolyzes 1 μmol of *N*-benzoyl-L-tyrosine ethyl ester (BTEE) per min at pH 7.8 and 25°C in 0.1 *N* NaCl solution. Enzyme activity was measured before the *in vitro* enzyme catalyzed biodegradation study.

Synthesis of Monomers and Polymers

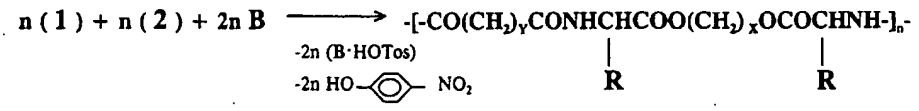
The general scheme of the synthesis of regular PEAs was divided into three major steps, and are shown in Schemes 1–3: the preparation of di-*p*-toluenesulfonic acid salts of bis (α-amino acid) α,ω-alkylene diesters (**1**), the preparation of di-*p*-nitrophenyl ester of dicarboxylic acids (**2**), and the polymer synthesis of PEAs (**3**) via solution polycondensation of (**1**) and (**2**).

Synthesis of Di-p-toluenesulfonic Acid Salts of Bis (α-amino acid) α,ω-Alkylene Diesters (1)

Di-*p*-toluenesulfonic acid salts **1** were prepared according to our previously published procedures[32] and are shown in Scheme 1. In principle, α-amino acid (2 mol) and diol (1 mol) were directly condensed in a refluxed benzene or benzene/nitrobenzene (1 : 1 v/v) mixture with the presence of *p*-toluenesulfonic acid monohydrate (2 mol). All of the salts, most of which were synthesized for the first time, were white crystalline products, and obtained in nearly quantitative yields (90–99%).



Scheme 2. Synthesis of di-*p*-nitrophenyl ester of dicarboxylic acids, **2**.



PEA, 3
y-(*)-AA-x

B - Acid acceptor;

y-(*)-AA-x is designated for PEAs, 3.

Where: **y** is the number of methylene groups in dicarboxylic acid residue.

(*), AA and **x** same as Scheme 1.

Scheme 3. Synthesis of PEAs, **3**, via solution polycondensation of di-*p*-toluenesulfonic acid salt, **1**, and di-*p*-nitrophenyl ester, **2**.

The salts **1** were purified by repeated recrystallization from water for three to four times.

Synthesis of di-p-nitrophenyl Ester of Dicarboxylic Acids (2)

As shown in Scheme 2, di-*p*-nitrophenyl adipate **2a** (m.p. 123–124°C) and di-*p*-nitrophenyl sebacate **2b** (m.p. 103–104°C) were prepared in nearly quantitative yields by the interaction of the corresponding dicarboxylic acyl chlorides (1 mol) with *p*-nitrophenol (2.01 mol) in organic solvents (e.g., ethylacetate, acetone, acetonitrile) in the presence of pyridine (2.01 mol). The resulting di-*p*-nitrophenyl ester of dicarboxylic acids **2** was purified by repeated recrystallization from ethylacetate. Their melting points were in agreement with the published data.[32]

Solution Polycondensation of (1) and (2)

As shown in Scheme 3, PEAs **3** were synthesized based on our previously published procedures.[25] In some cases, the reaction was carried out in a thermostat vessel equipped with a capillary, and the solution flow time was measured over a predetermined period for studying polycondensation rate. The vessel with a capillary was kept in a refrigerator at −20°C overnight. In other cases, the reaction vessels were kept overnight at 65°C in a thermostat oven without stirring. The resulting viscous reaction solutions were filtered through a glass filter and poured into distilled water. The precipitated tar-like masses were thoroughly washed with distilled water for 3–7 days at room temperature. After such a treatment, the tar-like substances were transformed into nonsticky solid or rubbery polymers that still contained residual *p*-nitrophenol, a low molecular weight by-product of the solution polycondensation. For making PEAs free of *p*-nitrophenol, the polymers were precipitated from a chloroform solution (10% v/w) into 15–20-fold excess (by volume) of ethylacetate. The precipitated polymers (fiber or powder appearance for those PEAs based on L-phenylalanine, and tar-like appearance for those PEAs based on other α-amino acids) were separated by decanting the liquid phase (ethylacetate + chloroform), washed for three to four times by fresh ethylacetate (40–50% of the starting volume of ethylacetate), and finally dried under a reduced pressure at 50–60°C up to constant weights. After drying, the tar-like polymers became corneous and were removed from the vessel by dissolving in chloroform and solvent casting onto glass plates. The chloroform was evaporated at room temperature, and the films obtained were dried at 60–65°C up to constant weights. The purity of the PEAs was checked by dissolving them (20 mg) in hot 10% NaOH solution (4 mL) and the absence of *p*-nitrophenoxide anion absorption at 430 nm would ensure the newly synthesized PEAs were free of residual *p*-nitrophenol.

Materials Characterization

The chemical structure of the materials synthesized were characterized by FTIR, UV-VIS, and

NMR spectrophotometers. A Nicolet Magna 560 FTIR spectrophotometer, coupled with a Nicolet data station with OMNIC 3.1 software with a resolution of 2 cm^{-1} and Specord UV-VIS (Carl Zeiss, Jena), were used for IR and UV-VIS analyses. The 400-MHz ^{1}H-NMR spectra (5 mm o.d. sample tubes) and 100-MHz ^{13}C-NMR spectra (5 mm o.d. sample tubes) were recorded on a Varian Unity 500 MHz spectrometer using solutions in $CDCl_3$ containing tetramethylsilane (TMS) as an internal standard. The molecular weights of the resulting PEAs (M_w, M_n and polydispersity) were determined by gel permeation chromatography (GPC) using a Kontron HPLC-420 instrument equipped with a Waters Associate differential refractometer (Model 410); GPC was carried out in tetrahydrofuran (THF) using polystyrene as the standard.

The thermal properties of these new materials were measured by Perkin–Elmer DSC-7 under a nitrogen gas flow. Because many of the PEAs were rather hydrophilic and could absorb atmospheric moisture during manipulations, samples were heated to remove the absorbed water, cooled, and reheated again at a heating/cooling rate of 20°C/min. The thermal data from the second DSC scan were used. Specific rotations $[\alpha]_D$ for **1** and **3** (sodium D-line) in deg · dm^{-1} · g^{-1} · cm^3 were measured at 25°C on a polarimeter SM-3 (ZOMZ, Zagorsk, Russian Federation) with cell length 1 = 10 cm, using DMA solutions and 2% (v/w) concentrations. The reduced viscosity (η_{red}) was determined in *m*-cresol at a concentration of 0.5 g/dL at 25°C. Thermomechanics were measured using puncheon with d = 4 mm at a loading 100 g and a heating rate of 2–3°C/min.

In Vitro Enzyme-Catalyzed Biodegradation Study

In vitro enzyme-catalyzed hydrolysis of PEAs was carried out by potentiometric titration (Radiometer RTS-822 titrator) at 37°C and pH 7.4 in 5 mL of 0.1 *N* NaCl solution in the presence of 2 mg of α-Chymotrypsin as described earlier.[25] PEA **3** films (about 200 mg with diameter = 5 cm) for these *in vitro* hydrolysis studies were cast from chloroform solutions. The solvent was evaporated at room temperature and PEA films were additionally dried at 65°C up to constant weights before use. The propensity of PEAs toward biodegradation was estimated by the amount of NaOH consumed per 70 min (one cycle of a "Radiometer RTS-822" titrator), which was used to neutralize the acidic carboxylic group released from the hydrolysis of the ester linkages in PEAs.

For studying whether there was any adsorption of enzymes (noncovalent immobilization) onto the PEA film surface and the effect of the immobilized enzyme on PEA biodegradation, the PEA film was removed from the enzyme solution after a 70-min incubation, thoroughly washed, with distilled water for five times, and placed again into a titrator cell containing fresh 0.1 *N* NaCl solution to determine the amount of NaOH consumed for another 70 min.

RESULTS AND DISCUSSION

Monomer Synthesis

In this study, there were total 12 different types of new di-*p*-toluenesulfonic acid salts of bis-(α-amino acid)-α,ω-alkylene diesters of the general formula **1** synthesized from the six α-amino acids and three α,ω-diols. The essential characteristics of these diamino-diester monomers are given in Table I. The L-Phe-3 and L-Phe-4 monomers were originally described in our previous publication.[25] The structures of the di-*p*-toluenesulfonic acid salt monomers **1** were confirmed by both elemental analysis and IR spectra. All the diamino-diester monomers **1** prepared from L-amino acids were optically active. All of the diamino-diester monomers had rather high melting temperatures (ranging from 190–264°C), typical for organic salts. The yields of the monomer synthesis ranged from 90–99%.

In 1979, Huang et al.[33] suggested a very simple and useful method for preparing α,α′-diamino diamino diesters like **1** by the direct condensation of L-phenylalanine with ethylene glycol in the presence of *p*-toluenesulfonic acid in refluxed benzene for 26 h. Our modification of this reaction,[25] based on the use of the nitrobenzene/benzene mixture (1 : 1 v/v) instead of pure benzene as a reaction medium, shortened the condensation time substantially (3 h vs. 26 h).

Di-*p*-toluenesulfonic acid salt monomers **1** are rather cheap monomers, and can be obtained in nearly quantitative yields based on our method. The monomers can easily be purified by recrystallization from water. These acid salt monomers contain two ester groups that can undergo either specific or nonspecific hydrolysis. They would be suitable as monomers for preparing linear heterochain biodegradable polymers like regular poly(ester amide)s[25] and regu-

Table I. Di-*p*-toluenesulfonic Acid Salts of Bis-(α-amino acid) α,ω-Alkylene Diesters

N	I,(*)-AA-x	Yield,[b] %	M.P.[c] °C	$[\alpha]_D$[d]	Empirical Formula (FW)	Elemental Analysis: Calculated C	H	N	S	Experimental C	H	N	S
1	L-Val-4	90	263–264	+15	$C_{28}H_{44}N_2O_{10}S_2$ (632.80)	53.15	7.01	4.43	10.13	53.38	7.23	4.21	10.08
2	L-Val-6	94	212–214	+16	$C_{30}H_{48}N_2O_{10}S_2$ (660.85)	54.53	7.32	4.24	9.70	54.04	7.69	3.74	10.09
3	L-Leu-3	93	227–229	+9	$C_{29}H_{46}N_2O_{10}S_2$ (646.83)	53.85	7.17	4.33	9.91	53.77	7.36	4.07	9.96
4	L-Leu-4	98	252–254	+10	$C_{30}H_{48}N_2O_{10}S_2$ (660.85)	54.53	7.32	4.24	9.70	54.85	7.41	4.14	10.02
5	L-Leu-6	99	190–192	+10	$C_{32}H_{52}N_2O_{10}S_2$ (688.91)	55.79	7.61	4.07	9.31	56.12	8.15	3.77	9.46
6	L-Ile 4	96	242–244	+20	$C_{30}H_{48}N_2O_{10}S_2$ (660.85)	54.53	7.32	4.24	9.70	54.49	7.39	4.03	9.61
7	L-Ile-6	98	198–201	+22	$C_{32}H_{52}N_2O_{10}S_2$ (688.91)	55.79	7.61	4.07	9.31	55.95	7.72	3.89	9.36
8	DL-Nle 4	97	216–218	0	$C_{30}H_{48}N_2O_{10}S_2$ (660.85)	54.53	7.32	4.24	9.70	54.71	7.38	4.10	9.84
9	L-Phe-3	96	255–257	+28	$C_{35}H_{42}N_2O_{10}S_2$ (714.86)	58.81	5.92	3.92	8.97	59.06	5.92	4.05	8.85
10	L-Phe-4	94	235–237	+30	$C_{36}H_{44}N_2O_{10}S_2$	9.32	6.08	3.84	8.80	59.34	6.34	3.56	8.55
11	DL-Phe-4	92	220–222	0	(728.89)					59.43	6.26	3.78	8.69
12	L-Phe-6	99	213–215	+31	$C_{38}H_{48}N_2O_{10}S_2$ (756.94)	60.30	6.39	3.70	8.47	60.63	6.86	3.60	8.56
13	DL-Met-4	92	211–213	0	$C_{28}H_{44}N_2O_{10}S_4$ (696.93)	48.26	6.36	4.02	18.40	48.37	6.29	3.89	18.48
14	DL-Met-6	95	190–193	0	$C_{28}H_{44}N_2O_{10}S_4$ (724.98)	49.70	6.67	3.86	17.69	49.69	6.93	3.58	17.83

[a] Absorption bands in the regions 1150–1160 cm^{-1} (—O—) and 1715–1735 cm^{-1} $-\overset{\overset{O}{\|}}{C}-$ are observed in the IR-spectra of the salts 1 obtained (in nujol).
[b] Yield of a raw product.
[c] Melting points after recrystallization from water.
[d] Specific rotation, in DMA, c = 2% (w/v), l = 10 cm.
Compounds N9 and N10 were described in our previous publication.[6j] Compounds N1–8, 11–14 were synthesized in this study.

lar poly(ester urethane)s[27] for biomedical applications because they are composed entirely of nontoxic building blocks. The acid salt monomers **1** could also be used as the crosslinking agents for preparing biodegradable networks and hydrogels,[34] which are considered as promising biomedical materials.[35–37]

Polymer Synthesis

Effect of Reaction Temperature, Duration, Molar Ratio, and Acid Acceptor on the Reaction Rate and Molecular Weight of PEAs 3

PEAs **3** were prepared according to the Scheme 3. The yields, viscosity characteristics, specific rotation $[\alpha]_D$, and data from elemental analysis of PEAs are given in Table II. Because of the fundamental relationship between molecular weight and properties of polymers, our primary goal in this study was to synthesize high molecular weight PEAs having useful material properties for biomedical applications. In our previous publication,[25] polycondensations of the salts like **1** with active diester **2a** were carried out in organic solvents [$CHCl_3$, *N*-methyl-2-pyrrolidinon (NMP)] in the presence of triethylamine as an acceptor for *p*-toluenesulfonic acid at room temperature. Relatively low molecular weight PEAs with $\eta_{red} < 0.6$ dL/g were obtained under those conditions. In the present work, we used monomers **1** (e.g., L-Phe-4) and **2a** as the example to illustrate the effects of reaction temperature and duration, the ratio of **1** to **2**, the nature of solvents, and type of acid acceptor **B** on the resulting molec-

Table II. Poly(ester amide)s 3 Obtained by Polycondensation of 1 with 2 According to Scheme 3[a)]

	Poly(ester amide) 3	Yield of	η_{red}			Elemental Analysis[b]							
						Calculated				Found			
N	y-(*)AA-x	3 in %	dL/g	$[\alpha]_D$	Empirical Formula (FW)	C	H	N	S	C	H	N	S
1	4-L-Val-4	98	1.60		$(C_{20}H_{34}N_2O_6)_n$ (398.50)n	60.28	8.60	7.03		60.53	8.72	7.18	
2	4-L-Val-6	95	0.69		$(C_{22}H_{38}N_2O_6)_n$ (426.56)n	61.95	8.98	6.57		61.12	8.92	6.67	
3	8-L-Val-4	99	2.55		$(C_{24}H_{42}N_2O_6)_n$ (454.61)n	63.41	9.31	6.16		63.50	9.19	5.91	
4	8-L-Val-6	98	1.71		$(C_{26}H_{42}N_2O_6)_n$ (482.66)n	64.70	9.61	5.80		64.79	9.82	5.64	
5	4-L-Leu-3	97	0.79		$(C_{21}H_{36}N_2O_6)_n$ (412.53)n	61.14	8.80	6.79		60.98	8.76	6.85	
6	4-L-Leu-4	97	1.58		$(C_{22}H_{38}N_2O_6)_n$ (426.56)n	61.95	8.98	6.57		61.79	9.06	6.79	
7	4-L-Leu-6	94	0.66		$(C_{24}H_{42}N_2O_6)_n$ (454.61)n	63.41	9.31	6.16		63.59	9.52	6.29	
8	8-L-Leu-3	98	2.03		$(C_{25}H_{44}N_2O_6)_n$ (468.64)n	64.07	9.46	5.98		64.18	9.70	5.80	
9	8-L-Leu-4	99	2.44		$(C_{26}H_{46}N_2O_6)_n$ (482.66)n	64.70	9.61	5.80		64.92	9.72	5.84	
10	8-L-Leu-6	100	2.99		$(C_{28}H_{50}N_2O_6)_n$ (510.72)n	65.85	9.87	5.49		65.88	9.75	5.60	
11	4-L-Ile-4	100	2.25		$(C_{22}H_{38}N_2O_6)_n$ (426.56)n	61.95	8.98	6.57		61.83	9.02	6.63	
12	4-L-Ile-6	99	1.34		$(C_{24}H_{42}N_2O_6)_n$ (454.61)n	63.41	9.31	6.16		63.01	9.17	6.59	
13	8-L-Ile-4	100	3.49		$(C_{26}H_{46}N_2O_6)_n$ (482.66)n	64.70	9.61	5.80		64.74	9.72	5.72	
14	8-L-Ile-6	100	3.37		$(C_{28}H_{50}N_2O_6)_n$ (510.72)n	65.85	9.87	5.49		65.61	9.81	5.58	
15	4-DL-Nle-4	99	2.60		$(C_{22}H_{38}N_2O_6)_n$ (426.56)n	61.95	8.98	6.57		61.76	8.71	6.45	
16	8-DL-Nle-4	100	3.08		$(C_{26}H_{46}N_2O_6)_n$ (482.66)n	64.70	9.61	5.80		64.58	9.53	5.78	
17	4-DL-Met-4	98	1.44		$(C_{20}H_{34}N_2O_6S_2)_n$ (462.63)n	51.92	7.41	6.06	13.86	51.79	7.60	6.26	14.06
18	4-DL-Met-6	95	0.57		$(C_{22}H_{38}N_2O_6S_2)_n$ (490.69)n	53.85	7.81	5.71	13.07	53.36	7.56	5.76	13.52
19	8-DL-Met-4	99	2.90		$(C_{24}H_{42}N_2O_6S_2)_n$ (518.74)n	55.57	8.16	5.40	12.36	55.69	8.26	5.61	12.50
20	8-DL-Met-6	99	2.05		$(C_{26}H_{46}N_2O_6S_2)_n$ (546.80)n	57.11	8.48	5.12	11.73	57.25	8.58	5.14	11.92
21	4-DL-Phe-4	99	1.29		$(C_{28}H_{34}N_2O_6)_n$ (494.56)n	68.00	6.92	5.66		68.12	6.84	5.78	
22	4-L-Phe-4	99	1.67	−24	$(C_{30}H_{38}N_2O_6)_n$ (494.56)n	68.00	6.92	5.66		68.24	6.72	5.78	
23	4-L-Phe-6	100	1.34	−23	$(C_{30}H_{38}N_2O_6)_n$ (522.64)n	68.94	7.33	5.36		68.80	7.52	5.14	
24	8-L-Phe-3	98	1.25	−22	$(C_{31}H_{40}N_2O_6)_n$ (536.67)n	69.38	7.51	5.22		69.14	7.36	5.40	
25	8-L-Phe-4	100	1.97	−23	$(C_{32}H_{42}N_2O_6)_n$ (550.70)n	69.79	7.69	5.09		69.83	7.73	5.35	
26	8-L-Phe-6	100	1.10	−22	$(C_{34}H_{46}N_2O_6)_n$ (578.75)n	70.56	8.01	4.84		70.92	8.36	5.02	

[a] PEAs are prepared under optimal reaction conditions (see text).

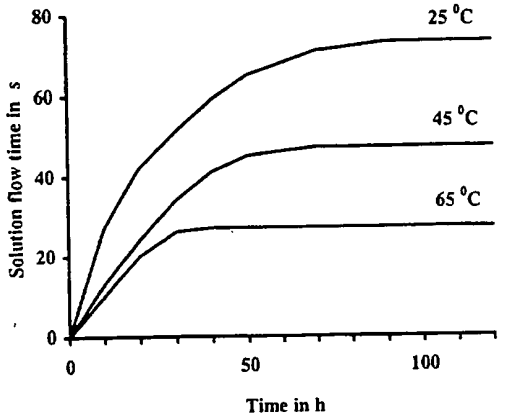


Figure 1. Kinetics of polycondensation of the salt **1** (L-Phe-4) with di-*p*-nitrophenyl adipate **2a** at various temperatures in DMA in the presence of NEt_3. Concentration of each monomer 0.6 mol/L, mole ratio **1** : NEt_3 = 1 : 2.1. Volume of NEt_3 has been taken into consideration.

ular weight of PEAs via solution polycondensation. It was found that the yields of PEA's were virtually quantitative in all cases studied.

As shown in Figure 1, the kinetic study of the polycondensation of **1** (L-Phe-4) and **2a** in *N,N*-dimethylacetamide (DMA) at room temperature (25°C) in the presence of NEt_3 as an acid acceptor revealed that the reaction proceeded too slowly and took 80–100 h to completion at a monomer concentration 0.6 dL/g. An increase in reaction temperature up to 65°C increased the polycondensation reaction rate and the reaction completed within 35–40 h. A further increase in the polycondensation temperature beyond 65°C, for example, to 85°C, however, led to an even higher rate of polycondensation at the expense of molecular weight of the resulting PEAs, as evident in the reduction of their η_{red}. Therefore, it appears that the optimal polycondensation reaction temperature for the highest η_{red} of PEA was 65°C.

The observed effect of reaction temperature on the rate of polycondensation of PEAs can be explained by the equilibrium between the salt **1** and the acid acceptor, NEt_3, in organic media as shown below:

$$-NH_2 \cdot HOTos + NEt_3 \leftrightarrows \sim NH_2 + NEt_3 \cdot HOTos \quad (1)$$

Due to the higher basicity of the resulting primary amines compared with NEt_3 in the medium of amidic type solvents,[38] the equilibrium could shift to the left, i.e., a reduction of the available free amine for its condensation with di-*p*-nitrophenyl ester of dicarboxylic acids, **2**. This suggests that, according to the above equilibrium reaction, the concentration of free amino groups generated in the reaction medium at a temperature < 65°C was not high enough for a faster rate of polycondensation.

The above-mentioned equilibrium reaction also illustrates the indispensable role of an acid acceptor, **B**, in the polycondensation reactions. This is because the acid acceptor led to the production of free amino groups from di-*p*-toluenesulfonic acid salts of bis (α-amino acid) α,ω-alkylene diesters, **1**, by removing TosOH from the acid salts, and the resulting free amino groups were required for the aminolysis of the active ester groups in **2** for the

Table III. Influence of the Acid Acceptor **B** on the Polycondensation of the Salt **1** (L-Phe-4) with di-*p*-Nitrophenyladipate **2a** (mol ratio 1/1) in DMA at 65°C and 48 h[a]

Acceptor (**B**)	Mole Ratio **B/1**	Appearance[b]	Yield of **3** in %	η_{red} dL/g (*m*-cresol)
NEt_3	2.1	**S**	99	0.97
NMM	2.1	**S**	98	0.87
TMED	1.05	**H**	99	0.95
K_2CO_3	1.05	**H**	95	0.51
K_2CO_3	2.1	**H**	96	0.75
K_2CO_3 + Ceolite 4 Å	1.05	**H**	99	1.17

[a] Concentration of each monomer 0.6 mol/L.
[b] S: homogeneous solution, H: heterogeneous mixture—K_2CO_3 is insoluble in DMA, TosOK, and TMED 2HOTos precipitated.

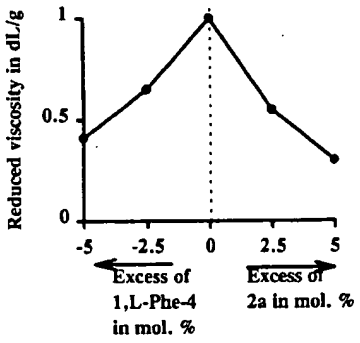


Figure 2. Dependence of reduced viscosity $\eta_{red.}$ of PEA **3** (4-L-Phe-4) on the monomers mol ratio. Polycondensation of the salt **1** (L-Phe-4) with di-*p*-nitrophenyl adipate **2a** in DMA at 65°C (duration 48 h). The concentration of each monomer at the point "0" concentration scale was 0.6 mol/L. Mol ratio **1** : NEt_3 = 1 : 2.1. Volume of NEt_3 has been taken into consideration.

formation of PEAs. Tertiary amines like triethylamine (NEt_3), *N*-methylmorpholine (NMM), and *N,N,N',N',*-tetramethylethylenediamine (TMED) were suitable acid acceptors in this reaction (Table III). The only difference among these acid acceptors lied in the solubility of the resulting salts in the reaction medium. For example, *p*-toluenesulfonic acid salts of NEt_3 and NMM were soluble in DMA, whereas the di-*p*-toluenesulfonic acid salts of TMED precipitated as crystals in DMA. This self-separation of low molecular weight byproducts would be desirable because it would facilitate the purification of the resulting PEAs. In addition, the precipitation of the salt byproducts would shift the equilibrium reaction (1) to the right, i.e., promoting polycondensation reaction. Inorganic salts like potassium carbonate can also be used as acid acceptors in these reactions. NEt_3 was chosen in this study because homogeneous reaction solutions were kept overnight at 65°C oven without stirring. In the case of heterogeneous systems (TMED, K_2CO_3), continuous stirring was necessary. Better results were also obtained by the use of molecular sieves 4 Å, which would absorb water released from the interaction of K_2CO_3 with TosOH. The polycondensation reaction in this case proceeded heterogeneously—both K_2CO_3 and the resulting TosOK were insoluble in DMA that would facilitate the purification of PEAs.

An equal molar ratio of the starting monomers **1** and **2** (Fig. 2) at a concentration 1.2 mol/L in the solution polycondensation was found to be the most appropriate for preparing high molecular weight PEAs.

Among the three solvents used ($CHCl_3$, *N*-methyl-2-pyrrolidinon, and DMA), DMA was found to be the best for synthesizing PEAs because the resulting PEAs have the highest reduced viscosity with less than one-half of the reaction time of chloroform as shown in Table IV.

Molecular Weight of PEAs 3

Based on the data presented above, we concluded that the optimal solution polycondensation conditions for synthesizing PEAs **3** in quantitative yields and excellent film-forming properties were: reaction temperature 65°C, duration 48 h, concentration of each monomer 1.2 mol/L, DMA as the reaction medium, and NEt_3 as an acid acceptor. The η_{red} of 4-L-Phe-4 PEA prepared under the above optimal polycondensation condition was as high as 1.67 dL/g vs. 0.6 dL/g obtained in our previous study.[25] All of other PEAs listed in Table II were also prepared under these optimal conditions, and their η_{red} varied from 0.66 (4-L-Leu-6) to 3.49 (8-L-Ile-4) dL/g. This wide range of molecular weight obtained appears to be attributed not

Table IV. Influence of the Reaction Medium on the Polycondensation of the Salt **1** (L-Phe-4) with di-*p*-Nitrophenyladipate **2a**[a] in the Presence of NEt_3

Solvent	*t* (°C)	Duration in h	Appearance[b]	Yield of **3** in %	η_{red} dL/g (*m*-cresol)
$CHCl_3$	25	100	S	99	0.38
N-MP	65	48	S	98	0.88
DMA	65	48	S	99	0.97

[a] Concentration of each monomer 0.6 mol/L, mol ratio 1 : 2 : NEt_3 = 1/1/2,1.
[b] S: homogeneous solution.

Table V. Thermal and Molecular Mass Characteristics of **PEAs 3**

Poly(ester amide) **3** y-(*)AA-x	(DSC)		(GPC)		
	T_g °C	T_m °C	M_w	M_n	M_w/M_n
4-L-Val-4	58				
4-L-Val-6	38				
8-L-Val-4	44	101			
8-L-Val-6	33		72,000	44,000	1.64
4-L-Leu-3	50		30,000	23,000	1.30
4-L-Leu-4	45		60,000	40,000	1.50
4-L-Leu-6	38		24,000	20,000	1.20
8-L-Leu-3	48		80,000	55,000	1.45
8-L-Leu-4	47				
8-L-Leu-6	37		137,000	88,000	1.56
4-L-Ile-4	47				
4-L-Ile-6	30		53,000	34,000	1.56
8-L-Ile-4	37				
8-L-Ile-6	28		167,000	107,000	1.56
4-DL-Nle-4	28				
8-DL-Nle-4	20		145,000	98,000	1.48
4-DL-Met-4	18				
4-DL-Met-6	11				
8-DL-Met-4	17				
8-DL-Met-6	13		38,000	21,000	1.81
4-DL-Phe-4	53		34,000	24,000	1.42
4-L-Phe-4	59	104			
4-L-Phe-6	49	124			
8-L-Phe-3	48	103			
8-L-Phe-4	47	111			
8-L-Phe-6	35	108			

GPC in tetrahydrofuran using polystyrene standards.
DSC in aluminum pans at a heating rate of 20°C/min under nitrogen. The results of the second heat are giver.

only to the difference in chemical structures of PEAs but also to the individual characteristics of the di-*p*-toluenesulfonic acid salts **1**, including their ability to reach different levels of grade purity for the polycondensation after the multiple (three to four times) recrystallization from water.

The GPC measurements were carried out for several selected PEAs in THF and the data are given in Table V. PEAs having molecular weight as high as 167,000 (M_w varies from 24,000 to 167,000) and narrow polydispersity (M_w/M_n from 1.20 to 1.81) had been made in this study. Good correlations between the η_{red} of PEAs in *m*-cresol and M_w (correlation coefficient $r = 0.9968$) and M_n ($r = 0.9899$) have been found as shown in Figure 3. This suggests that molecular weights of PEAs **3** (at least for PEAs containing side alkyl substituents **R**) can be calculated precisely from the measurements of their η_{red}.

Chemical Structure Identification of PEAs 3

The structure of PEAs **3** was confirmed by both elemental analysis (Table II) and FTIR spectra. Figure 4 shows the FTIR spectra of the representative PEAs, 4-L-Phe-4, 8-L-Phe-6, 4-L-Leu-4, and 8-L-Leu-6. The carbonyl bands at 1648–1650 cm^{-1} (amide I), 1538–1542 cm^{-1} (amide II), and 1738–1742 cm^{-1} (ester), and NH vibrations at 3290 cm^{-1} are typical for all PEAs obtained. The 4-L-Phe-4 and 8-L-Phe-6 samples had two additional characteristic bands at 700 and 750 cm^{-1} due to the presence of phenyl group. The corresponding ^{1}H-NMR and ^{13}C-NMR spectra of these four representative PEAs are given in Figures 5 and 6, respectively. The ^{1}H-NMR spectra showed the expected NH, aromatic, and aliphatic signals, and confirmed the expected structure. The two >C=O signals shown in the ^{13}C-NMR spectra

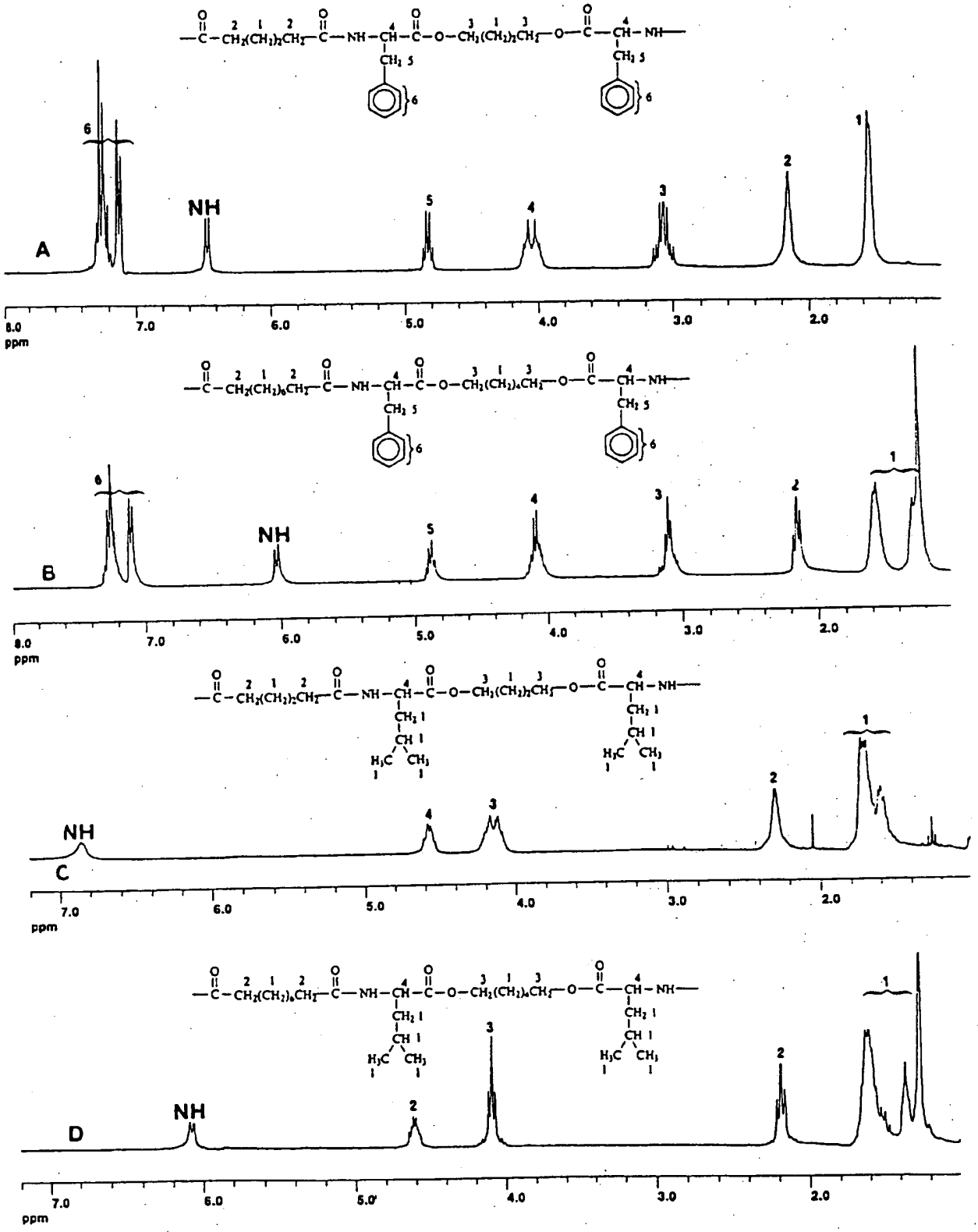


Figure 5. ^{1}H-NMR spectra of four representative PEAs **3**. (A) 4-L-Phe-4; (B) 8-L-Phe-6; (C) 4-L-Leu-4; (D) 8-L-Leu-6 (300 MHz, $CDCl_3$).

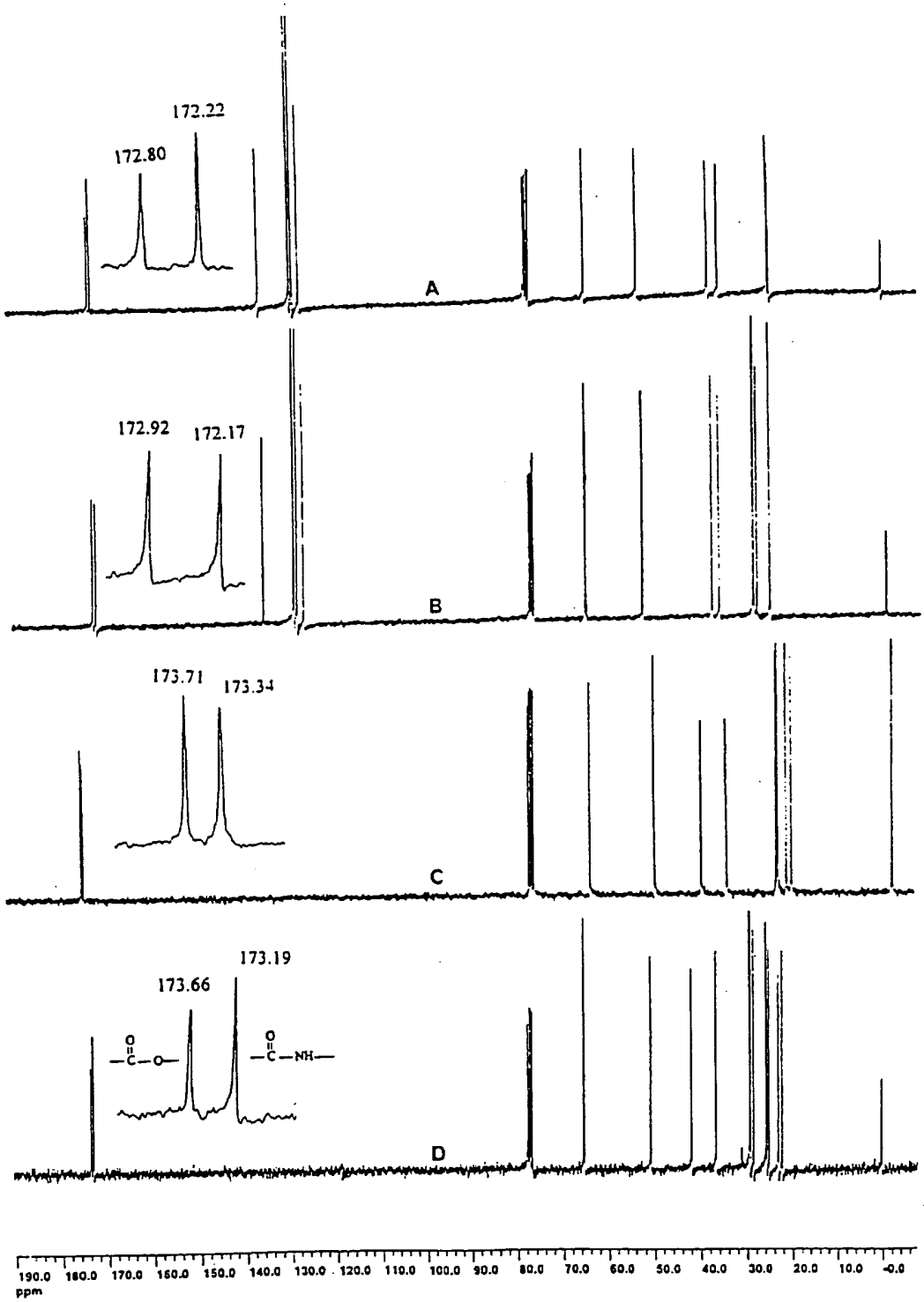


Figure 6. ^{13}C-NMR spectra of four representative PEAs **3.** (A) 4-L-Phe-4; (B) 8-L-Phe-6; (C) 4-L-Leu-4; (D) 8-L-Leu-6 (100 MHz, $CDCl_3$).

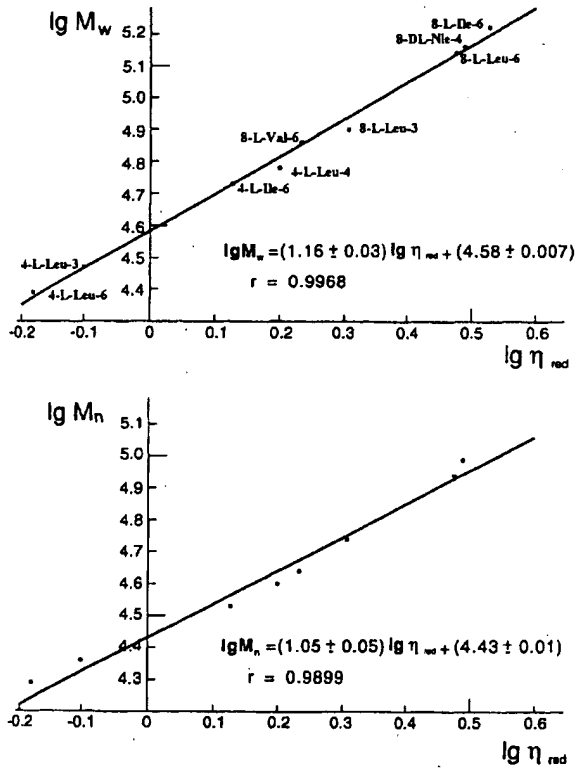
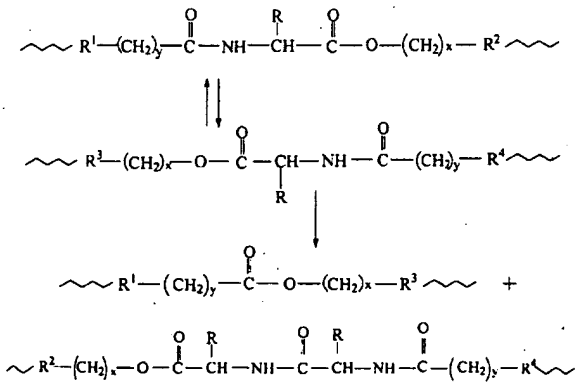


Figure 3. Linear relationship between weight (M_w) in number (M_n) average molecular weight of PEAs **3** and their reduced viscosity, η_{red},

(Fig. 6) are typical for all the four representative PEAs and were in favor of a regular structure of PEAs without the interchange reaction between amide and ester groups taking place during the specified synthesis method. If there were interchange reaction between amide and ester groups, there would be more than two >C═O signals because other types of ester and amide groups would be formed and lead to the formation of irregular PEA backbones, as illustrated below:



Thermal Properties of PEAs 3

As shown in Table V, the glass transition temperature, T_g, of the newly synthesized PEAs **3** ranged from 11 to 59°C, and most of them were amorphous, except those PEAs made from L-phenylalanine [y-(*)-Phe-x] and some L-valine that exhibited semicrystalline characteristic with melting temperature, T_m, in the low 100s°C. An examination of the effect of the polymethylene chain length in diols (x) and in dicarboxylic acid (y) on T_g of PEAs revealed that an increase in polymethylene chain length reduced T_g as expected, and the effect of change x in diols on T_g was more profound than y in dicarboxylic acids. For example, an increase of x by 2 (e.g., from butanediol to hexanediol) reduced T_g more than an increase of y by 4, as shown in 4-L-Val-4/4-L-Val-6 (ΔT_g = 20°C) vs. 4-L-Val-4/8-L-Val-4 (ΔT_g = 14°C), 4-L-Ile-4/4-L-Ile-6 (ΔT_g = 17°C) vs. 4-L-Ile-4/8-L-Ile-4 (ΔT_g = 10°C), and 4-DL-Met-4/4-DL-Met-6 (ΔT_g = 7°C) vs. 4-DL-Met-4/8-DL-Met-4 (ΔT_g = 1°C).

To elucidate the influence of α-amino acid residues on T_g, PEAs with equal x and y are listed together in Table VI. An analysis of these data concludes that those PEAs based on DL-amino acids had a lower T_g than the PEAs based on the corresponding L-isomers (e.g., 4-DL-Phe-4 vs. 4-L-Phe-4). Among those PEAs that consisted of L-amino acids, the PEAs synthesized from L-amino acids with symmetrical side substituents like Val, Leu, and Phe had a higher T_g than those PEAs from nonsymmetrical side substituents like Met and Ile. This substituent effect was more pronounced at higher x and y values (e.g., PEAs

Table VI. The Influence of α-Amino Acids' Nature on T_g of PEAs

PEA 3	T_g, °C	PEA 3	T_g, °C
4-L-Val-4	58	8-L-Val-4	44
4-L-Leu-4	45	8-L-Leu-4	47
4-L-Ile-4	47	8-L-Ile-4	37
4-DL-Nle-4	28	8-DL-Nle-4	20
4-DL-Met-4	18	8-DL-Met-4	17
4-L-Phe-4	59	8-L-Phe-4	47
4-DL-Phe-4	53		
4-L-Val-6	38	8-L-Val-6	33
4-L-Leu-6	38	8-L-Leu-6	37
4-L-Ile-6	30	8-L-Ile-6	28
4-DL-Met-6	11	8-DL-Met-6	13
4-L-Phe-6	49	8-L-Phe-6	35

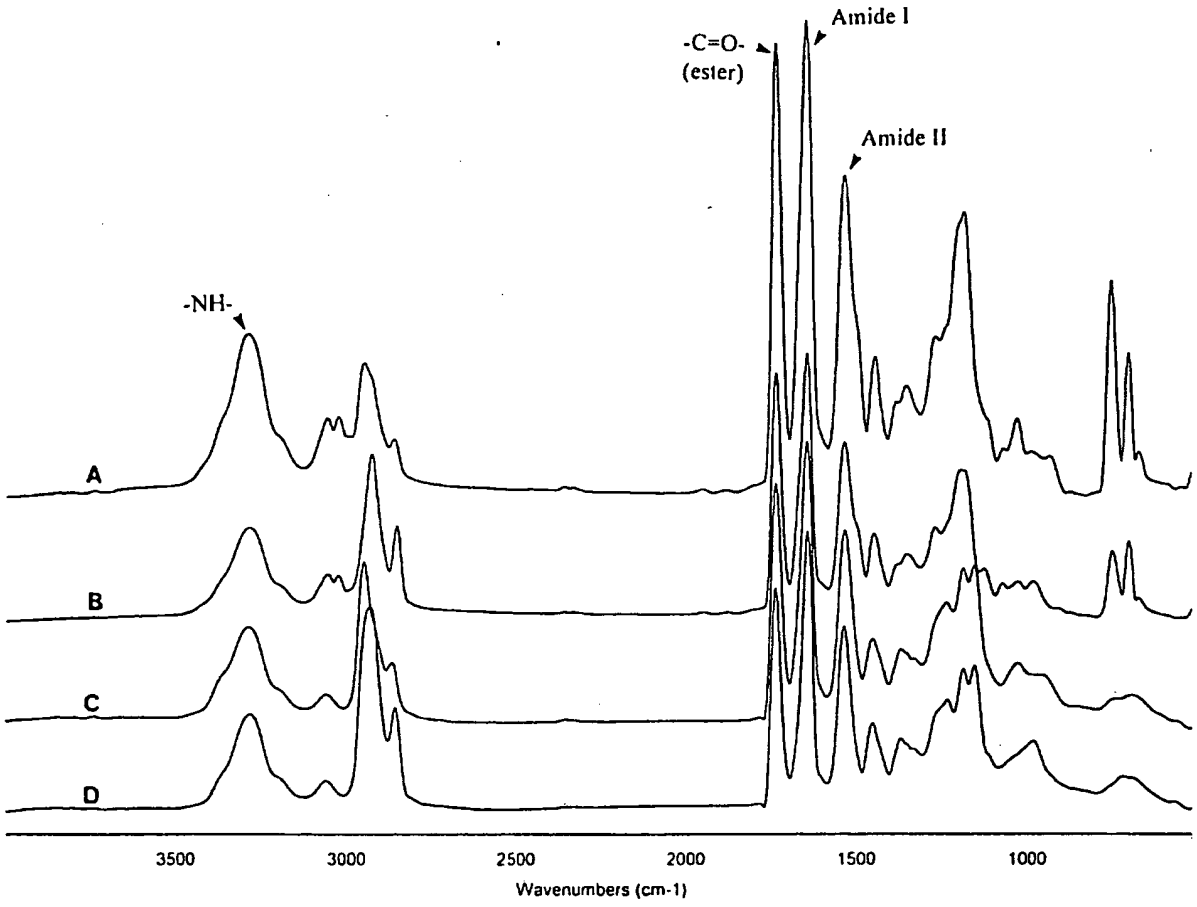


Figure 4. FTIR spectra of four representative PEAs **3**. (A) 4-L-Phe-4; (B) 8-L-Phe-6; (C) 4-L-Leu-4; (D) 8-L-Leu-6.

based on Leu and Ile), and became less apparent with smaller of x and y. The PEAs based on α-amino acids having aromatic substituents like L-Phe always had the highest T_g, while the DL-Met-based PEAs had the lowest T_g. The spread in T_g values, ΔT_g, between the highest and lowest within a group of PEAs having the same x and y became smaller when the values of x and y became large. For example, the ΔT_g was 41°C when x and y were 4, and 22°C when x was 6 and y was 8.

The thermomechanical data of PEAs suggest that these new PEAs had relatively low fusion temperature from 20 to 130°C. This low fusion temperature would be advantageous for using these polymers for drug control/release devices.

Solubility

The solubility of PEAs (0.1 g) in common organic solvents (1.0 mL) at room temperature was assessed. All of the PEAs **3** synthesized were soluble in a common organic solvent like chloroform, but did not dissolve in ethyl acetate, as shown in Table VII. PEAs composed of valine and leucines dissolved in methanol, ethanol, and tetrahydrofuran. Except those semicrystalline PEAs based on L-Phe, amorphous PEAs were corneous materials in a dry state, and became pliable after swelling in water. All the polymers were able to form flexible films from the solutions. PEAs based on L-phenylalanine had fiber-forming properties as well.

Preliminary In Vitro *Biodegradation Study*

Table VIII summarizes the preliminary results of the α-chymotrypsin catalyzed hydrolysis of the newly synthesized PEAs **3** for an interval of 70 min for both the dissolved and surface-immobilized enzyme. Among all PEAs synthesized, those PEAs based on L-phenylalanine showed the high-

Table VII. Solubility of PEAs in Organic Solvents at Room Temperature[a]

PEA 3	Organic Solvent						
y-(*)AA-x	Ethyl Acetate	Methanol	Ethanol	Diethyl Ether	Chloroform	Dioxane	Tetrahydrofuran
4-L-Val-4	−	+	±	−	+	−	−
4-L-Val-6	−	+	+	−	+	−	−
8-L-Val-4	−	+	+	−	+	−	−
8-L-Val-6	−	+	+	−	+	−	+
4-L-Leu-3	−	+	+	−	+	+	+
4-L-Leu-4	−	+	+	−	+	+	+
4-L-Leu-6	−	+	+	−	+	+	+
8-L-Leu-3	−	+	+	−	+	±	+
8-L-Leu-4	−	+	+	−	+	+	+
8-L-Leu-6	−	+	+	−	+	+	+
4-L-Ile-4	−	+	+	−	+	+	+
4-L-Ile-6	−	+	+	−	+	+	+
8-L-Ile-4	−	+	+	±	+	+	+
8-L-Ile-6	−	+	+	−	+	+	+
4-DL-Nle-4	−	+	+	−	+	−	−
8-DL-Nle-4	−	+	+	−	+	−	+
4-DL-Met-4	−	−	−	−	+	−	−
4-DL-Met-6	−	−	−	−	+	−	−
8-DL-Met-4	−	−	−	−	+	−	−
8-DL-Met-6	−	−	−	−	+	−	+
4-DL-Phe-4	−	−	−	−	+	−	−
4-L-Phe-4	−	−	−	−	+	−	−
4-L-Phe-6	−	−	−	−	+	−	−
8-L-Phe-3	−	−	−	−	+	−	−
8-L-Phe-4	−	−	−	−	+	−	−
8-L-Phe-6	−	−	−	−	+	−	−

(+): Soluble; (−): Insoluble; (±): Partially soluble or swells.
[a] 0.1 g PEA in 1.0 mL of solvent at 25°C.

est tendency toward the α-chymotrypsin–catalyzed hydrolysis, while the hydrolysis of the DL-isomer occurred at a lower rate than the L-isomer. The high hydrophobicity of the benzyl side groups in the phenylalanine-based PEAs was suggested to be responsible for this finding.[39] As we demonstrated in our previous work,[25] an increase in hydrophobicity due to the lengthening of the methylene groups in diol (x) led to the acceleration of α-chymotrypsin–catalyzed hydrolysis of the PEAs. In this study, we found that an increase in both x of diols and y of dicarboxylic acids also increased the sensitivity of PEAs toward the α-chymotrypsin–catalyzed hydrolysis. In addition to the hydrophobicity factor, the change in chain mobility due to different x and y may also be the factor that contributes to the different rate of enzyme-catalyzed hydrolysis of PEAs.

Following L-phenylalanine, DL-methionine– and L-leucine–based PEAs also exhibited relatively high tendency toward the α-chymotrypsin–catalyzed hydrolysis. The DL-norleucine–based PEAs also showed some tendency toward the α-chymotrypsin–catalyzed hydrolysis, but their rate was not as high as it was anticipated on the basis of Klyosov et al.'s findings of similar low molecular weight substrates (alkyl esters of *N*-acyl-norleucine).[47] This discrepancy might be related to the racemic nature of the amino acid and the observed film's contraction for some PEAs as well. We found that valine-, leucine-, and methionone-based PEAs film samples contracted after immersion in water and, hence, the surface area of the film changed with time during the *in vitro* α-chymotrypsin–catalyzed hydrolytic study. Therefore, the results from the valine-, leucine-, and methionone-based PEAs as well as 8-L-Phe-3, which had the unusually lowest α-chymotrypsin–catalyzed hydrolytic rate among the L-phenylalanine–based PEAs, must be considered as preliminary.

Table VIII. Preliminary Data on α-Chymotrypsinolysis of PEAs 3. Film Ø = 5 cm m = 200 mg, Cast from Chloroform Solution. Potentiometric Titration, 0.1 N NaCl pH = 7.4 t = 37°C.

	Enzyme in Solution (2 mg/5 mL)	Surface Immobilized Enzyme
y-(*)AA-x	NaOH consumption in μmol per 70 min	
4-L-Val-4	0	0
4-L-Val-6	0	0
8-L-Val-4	0	0
8-L-Val-6	0	0
4-L-Leu-3	26.5	5.7
4-L-Leu-4	31.0	1.6
4-L-Leu-6	6.0	2.6
8-L-Leu-3	12.6	3.5
8-L-Leu-4	7.1	4.8
8-L-Leu-6	7.5	3.9
4-L-Ile-4	0	0
4-L-Ile-6	0	0
8-L-Ile-4	0	0
8-L-Ile-6	0	0
4-DL-Nle-4	9.4	2.3
8-DL-Nle-4	3.2	0
4-DL-Met-4	28.4	0
4-DL-Met-6	7.2	0
8-DL-Met-4	7.7	2.5
8-DL-Met-6	2.2	0
4-DL-Phe-4	6.9	2.1
4-L-Phe-4	27.0	14.9
4-L-Phe-6	23.0	17.8
8-L-Phe-3	8.1	6.0
8-L-Phe-4	28.0	7.5
8-L-Phe-6	40.5	12.1

The L-valine–based PEAs, which contained a shorter and less hydrophobic side group R, and the L-isoleucine–based PEAs that contained sterically a more hindered side group R, showed nondetectable hydrolytic degradation activity toward the α-chymotrypsin–catalyzed hydrolysis under the testing condition.

Based on the results obtained in this study, we qualitatively conclude that the general tendency of the newly synthesized regular PEAs toward the α-chymotrypsin–catalyzed hydrolysis is consistent with Hansch's constants used to characterize the hydrophobicity of the side substituent R of α-amino acids and the effectiveness of the interaction of the side groups with the enzyme's hydrophobic pocket. The higher the overall hydrophobicity of the polymer backbone is, the more sensitive the polymer toward the enzyme-catalyzed hydrolysis is.

As to the hydrolysis of PEAs under the spontaneous immobilization of α-chymotrypsin onto the PEA surface, the L-phenylalanine-based PEAs had again the most tendency toward enzyme catalyzed hydrolysis, followed by L-leucine–based and some DL-norleucine– and DL-methionine–based PEAs. This general tendency was the same as the enzymatic hydrolysis in the solution case.

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REFERENCES AND NOTES

1. Törmälä, P. Adv Mater 1992, 4, 589.
2. Vainiopää, S.; Rokkanen, P.; Törmälä, P. Prog Polym Sci 1989, 14, 679.
3. Nathan, A.; Kohn, J. In: Biomedical Polymers: Design-to-Degrade System, Shalaby, S. W., Ed.; Hanser publishers: Munich, 1994; p. 117.

4. Drobnik, J. Adv Drug Delivery Rev. 1989, 3, 229.
5. Sadesh Kumar, G. Biodegradable Polymers—Prospects and Progress, Marcel Dekker, Inc.; New York, 1987.
6. Sela, M.; Katchalski, E.; Olitzki, A. L. Science 1956, 123, 1129.
7. Stewart, F. H. C. Aust J Chem 1969, 22, 1291.
8. Ridge, B.; Rydon, H. N.; Snell, C. R. J Chem Soc Perkin I, 1972, 2041.
9. Goodman, M. J Polym Sci Polym Symp 1978, 62, 173.
10. Kunz, H.; Lorenz, K. Angew Chem 1980, 92, 953.
11. Yoshida, M.; Asano, M.; Kumakura, M. Makromol Chem Rapid Commun 1990, 11, 337.
12. Shalaby, S. W.; Koelmel, D. F. U.S. Pat. (to Ethicon, Inc.), No. 4,441,496 1984.
13. Int' Veld., P. J. A.; Shen, Z. R.; Takens, G. J. A.; Dijkstra, P. J.; Feijen, J. J Polym Sci Part A: Polym Chem 1994, 32, 1063.
14. Yonezava, N.; Toda, F.; Hasegava, M. Makromol Chem Rapid Commun 1985, 6, 607.
15. Ouchi, T.; Nozaki, T.; Okamoto, Y.; Shiratani, M.; Ohya, Y. 5th SPSJ International Polymer Conference (IPC 94) Osaka, Japan, Nov–Dec. 1994.
16. Barrera, D. A.; Zylstra, E.; Lansbury, P. T. Jr.; Langer, R. J Am Chem Soc 1993, 115, 11010.
17. Zaalishvili, M. M.; Katsarava, R. D.; Rogozhin, S. V.; Davidovich, Yu. A.; Kart-velishvili, T. M.; Patsuria, M. M.; Kharadze, D. P. Pat. USSR, No 803417, 1980.
18. Zaalishvili, M. M.; Katsarava, R. D.; Kartvelishvili, T. M. Pat. USSR, No 948113, 1982.
19. Katsarava, R. D.; Kartvelishvili, T. M.; Davidovich, Yu. A.; Zaalishvili, M. M.; Rogozhin, S. V. Dokl Akad Nauk SSSR, 1982, 266, 363.
20. Katsarava, R. D.; Kartvelishvili, T. M.; Zaalishvili, M. M Bull Acad Sci Georgian SSR, 1984, 113, 533.
21. Katsarava, R. D.; Kharadze, D. P.; Kirmelashvili, L. I.; Zaalishvili, M. M. Acta Polym., 1985, 36, 2.
22. Katsarava, R. D.; Kharadze, D. P.; Japaridze, N.; Avalishvili, L. M.; Omiadze, T. N.; Zaalishvili, M. M. Makromol Chem 1985, 186, 939.
23. Katsarava, R. D.; Kharadze, D. P.; Japaridze, N. Sh. Vysokomolek. Soed.: Ser. B, 1986, 28, 518.
24. Katsarava, R. D.; Kharadze, D. P.; Kirmelashvili, L. I.; Medzmariashvili, N. G.; Goguadze, Ts. A.; Tsitlanadze, G. V. Macromol Chem 1993, 194, 143.
25. Arabuli, N.; Tsitlanadze, G.; Edilashvili, L.; Kharadze, D.; Goguadze, Ts.; Beridze, V.; Gomurashvili, Z.; Katsarava, R. Macromol Chem Phys 1994, 195, 2279.
26. Kartvelishvili, T. M.; Kvintradze, A.; Katsarava, R. Macromol Chem Phys 1996, 197, 249.
27. Kartvelishvili, T.; Tsitlanadze, G.; Edilashvili, L.; Japaridze, N.; Katsarava, R. Makromol Chem Phys 1997, 198, 1921.
28. Pratt, L. M.; Chu, C. C. J Polym Sci Part A: Polym Chem 1994, 32, 949.
29. Greisler, H. P.; Dennis, J. W.; Endean, E. D.; Ellinger, J.; Freisler, R.; Burgess, W. H. J Vasc Surg 1989, 9, 588.
30. Greisler, H. P.; Henderson, S. C.; Lam, T. M. J Biomater Sci: Polym Ed 1993, 4, 415.
31. Kohn J.; Langer, R. J Am Chem Soc 1987, 109, 817.
32. Zahn H.; Schade, F. Chem Ber 1963, 96, 1747.
33. Huang, S. J.; Bansleben, D. A.; Knox, J. R. J Appl Polym Sci 1979, 23, 429.
34. Kartvelishvili T.; Katsarava, R. The 2nd Far-Eastern Symposyum on Biomedical Materials, Oct. 4–6, 1995; Kyoto. Abstract, P. 37.
35. Bruin, P.; Veenstrs, G. L.; Nijenhuis, A. J.; Pennings, A. J. Makromol Chem Rapid Commun 1988, 9, 589.
36. Storey, R. F.; Wiggins, J. S.; Puckett, A. D. J Polym Sci Part A: Polym Chem 1994, 32, 2345.
37. Vyavahare N.; Kohn, J. J Polym Sci Part A: Polym Chem 1994, 32, 1271.
38. Madic C.; Tremillon, B. Bull Soc Chim France, F-4, N T-4, 1634, 1968.
39. Hansch, C. J Org Chem 1972, 37, 92.
40. Klyosov A. A.; Berezin, I. V. Fermentativnyi Kataliz (Enzymatic Catalysis), part 2, Moscow University Publ., Moscow, 1980, p. 114.

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